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Growth performance of Nile tilapia, *Oreochromis niloticus* (Linn.) in relation to provision of substrate and supplementary feeding, and grown in brackishwater ponds

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Abstract

The present study attempts to assess the effects of periphyton and supplementary feeding with commercial pelleted feeds on the growth performance of juvenile Nile tilapia, *Oreochromis niloticus* (mean body weight 1.49g) grown in inland brackish groundwater ponds for 90 days. Three treatments (with bamboo substrate grown with periphyton; with supplementary feeding without bamboo substrate; control – neither bamboo substrate nor supplementary feeding) were tested in duplicate in brackish water ponds. Irrespective of the treatments, all ponds were fertilized with cowdung at 7500 $kg \cdot ha^{-1} \cdot yr^{-1}$. Increase in the growth of fish reared in ponds provided with substrate was significant ($P<0.05$), about 31 and 56% higher than those in supplementary feeding and control ponds respectively. Length-weight relationship ($W=cL^n$) also showed higher exponential value (n) of length in substrate ponds (n=3.1) compared to other treatment ponds. Among the water quality parameters tested, the calcium content, turbidity, NH_4-N, NO_2-N, net primary productivity, phytoplankton, chlorophyll a and epilithic periphyton density were significantly ($P<0.05$) lower in substrate ponds compared to other treatment ponds; no significant differences in other water quality parameters were observed among all the treatment ponds. Significantly ($P<0.05$) higher values of periphyton biomass were observed at a depth of 50 cm, while autotrophic index remained lower at this depth in substrate ponds. Sediment chemistry indicated significantly ($P<0.05$) higher values for

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alkalinity, NO_3-N, organic matter and benthos in supplementary feeding ponds compared to other treatment ponds Fish grown in ponds provided with additional substrate had significantly ($P<0.05$) higher values of digestive enzyme activity (protease, amylase and cellulolytic). High accumulation of muscle protein, muscle glycogen and higher values of viscero-somatic index and hepato-somatic index were also observed in fish grown in substrate ponds as compared to others. Results of the present study clearly suggest that provision of bamboo substrate colonized with periphyton could significantly enhance the growth and yield of Nile tilapia grown in brackish groundwater ponds. The presence of periphyton in culture ponds is thus useful to enhance growth of fish low in the food web such as the tilapia species.

Introduction

Tilapias are considered to be one of important food fishes and thus are cultured/grown all over the world, as they are hardy and tolerate wide range of salinities and very low levels of dissolved oxygen (<0.5 $mg \cdot L^{-1}$). Although tilapias are regarded as omnivorous, a considerable body of evidences suggests that they are primarily herbivorous (Trewavas 1983). Stomach content analysis has revealed that they feed mainly on algae and algal based detritus (Trewavas 1983; Getachew 1987; Kumar et al. 2005). They can also feed directly on suspended algae, however, the quantity of the algae ingested in this way is probably not sufficient to fully meet their energy demands (Dempster et al. 1995). These species perhaps require other food sources, such as benthic algae, algal detritus or plant fodder to meet their energy requirements (Dempster et al. 1993; Yakupitiyage 1993). Laboratory studies by Dempster et al. (1995) have demonstrated that algal ingestion rates by tilapias are much greater when food is presented as a periphytic mat than when presented as dispersed planktonic organisms Huchette et al. (2000) have also demonstrated that tilapias graze efficiently on periphyton communities grown on submerged artificial substrate in cages. Verdegem et al. (2001) through analyzing the proximate composition of the stomach content of tilapia have shown that the periphyton has higher nutritive value under ungrazed (protein 41.4% and fat 7.9%) than grazed conditions (protein 23-26% and fat 2.7%). Enhancement of periphytic biomass in aquatic systems has been shown to increase fish growth/production significantly especially of those species which thrive low in the food chain both under freshwater (Azim et al. 2002) as well as in stagnant inland saline groundwater ponds (Jana et al. 2004; 2006).

Although studies have demonstrated that tilapias when grown in ponds fertilized with cow-dung respond very well to artificial/ supplemen-

tary diets (Bardach et al. 1972; Boyd 1982), their response to artificial substrates provided in ponds for enhancing periphytic biomass has not been well documented. Studies by Keshavanath et al. (2002) and Azim et al. (2002; 2004) have demonstrated that the provision of substrate could also reduce the need for artificial feed in freshwater fish ponds and thus can be an alternative to supplementary feeding in the culture of herbivorous fish species. Though, it is well known that periphyton forms an important food source in brackishwater fish farming and found to enhance primary production, only limited information on the role of periphyton in fish production from coastal areas (Hem and Avit 1994; Konan-Brou and Guiral 1994) is available. In the present study, we investigated the effect of periphyton on growth performance of Nile tilapia in inland brackish groundwater ponds and compared the effect of substrate with that of supplemental feeding. In addition, the impacts of periphyton on the fish's hepato-somatic index (HIS), viscera-somatic index (VSI), digestive enzyme activities and carcass composition are investigated. Hydrobiological parameters of the pond water and sediments are also monitored during the course of the experiment.

Materials and Methods

The experiment was conducted in six earthen ponds (15m x 25m x 1.5m each) located in the brackishwater research station of the Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar, India in March-June 2004. All experimental ponds were sun-dried and cleared off all vegetation prior to the commencement of the experiment. Bamboo poles (1.0m x 3.1cm diameter each) planted vertically in two of the experimental ponds were used as substrates for developing the periphyton as natural feed for the fish. In each of these two ponds, about 299 bamboo substrates or poles (each was vertically planted with 80 cm of it above the pond bottom) were erected at equal distance of one meter from one another, providing an additional total submerged surface area of about 29.1 m^2. All experimental ponds were further applied with quick lime (CaO) at 200 kg•ha^{-1} followed by fertilization with cow dung before the ponds are filled with brackishwater pumped from deep aquifers. Water salinity during the experimental period in all ponds fluctuated between 12.8 to 13.5 ppt.

In all experimental ponds, cow dung at a dose of 11.7 kg•pond^{-1} (375 m^2) and diluted with pond water at 1:3 w/v was applied to fertilize the pond. The experiment was carried out with the following treatments, each treatment conducted in duplicate ponds:

Treatment 1: Bamboo substrate was provided; no supplementary feeding with commercial pelleted feed was attempted.

Treatment 2: No bamboo substrate was provided; supplementary feeding with commercial pelleted feed was attempted.

Treatment 3: Neither bamboo substrate nor supplementary feeding with commercial pelleted feed were provided.

Stocking

Two weeks after the application of the first dose of organic fertilizer (on March 21, 2004), 30 days old tilapia (*Oreochromis niloticus*) with average body weight of 1.49 g•fish^{-1} were stocked at a density of 10,000 fish•ha^{-1}. The fish were grown for 90 days during the experiment.

Feeding

The fish in treatment 2 (supplementary feeding) were fed at 5% BW•day^{-1} in two feedings at 08:00-09:00 h and another at 15:00-16:00 h, a commercial pelleted feed using the 40% crude protein fish meal as the main ingredient (chemical composition: fat – 7.4%; crude fiber – 5.8%; nitrogen free extract – 35.8%; ash – 11.0% and energy - 18.5 kJ g-^{1}). Feeding rate was adjusted every 15th day after weighing a representative sample of about 80-100 fish.

Determination of periphyton biomass

In all experimental ponds, the periphyton biomass from the bamboo substrates was determined on day 15 after the ponds were fertilized with cow dung and, thereafter, on biweekly basis. Both the dry matter (DM) and pigment (chlorophyll a and phaeophytin a) methods as described by APHA (1998) were employed to determine the periphyton biomass. The periphyton from the bamboo substrates was sampled following the method described by Azim et al. (2001a), which involved in taking two 3cm x 3 cm samples of periphyton at each of the four water depths (0, 25, 50 and 75 cm at and below the water surface) per bamboo substrate; periphyton samples were taken from three randomly selected bamboo substrates in each of the experimental ponds

One from the two replicate samples of periphyton in each water depth of each bamboo substrate in each experimental pond was used to determine the dry matter (DM), ash free dry matter (AFDM) and ash contents as well as to calculate autotrophic index (AI) following the procedures described by APHA (1998).

AI was calculated as follows:

AI =[Biomass (ash-free weight of organic matter, mg•m^{-2})]/Chlorophyll *a*, mg•m^{-2}

Ash values were used to calculate periphyton productivity (APHA 1998) and expressed as follows:

Periphyton productivity (mg ash free weight •m^{-2}•d^{-1}) = [Ash free weight (mg•cm^{-2})]×100 / t

Where, t = duration of experiment (90 days).

Out of the remaining three samples of each replicate per depth, two were used for determining periphyton population. Samples from each depth were suspended in 50 ml of distilled water and stored in plastic bottles. Periphyton number was enumerated using a Sedgwick-Rafter cell according to the procedures used for determining the plankton population and calculated as follows:

$$N = P \times C \times 100/S$$

Where, N = periphyton number cm^{-2} (whether single celled or multi cellular, counted as one unit)

P = total number of periphyton units counted in 10 fields of Sedgwick-Rafter cell

C = volume of final concentrate sample (ml)

S = area of scraped surface (cm^2)

The remaining one sample from each replicate was used to determine chlorophyll *a* and pheophytin *a* contents following the methods described by APHA (1998).

From each treatment, for comparison, periphyton samples (in replicate of four 3×3 cm^2 samples) growing on the pond walls (epilithic) were also taken twice (at 45 day interval) during the experimental period of 90 days for the study of periphyton population and pigment concentrations.

Water quality monitoring

Water samples in replicate of four were obtained from each pond (i.e. 8 samples from each treatment) before sunrise at 45 day interval (on May 5, 2004 and June 20, 2004) for the study of physico-chemical characteristics (electrical conductivity, dissolved oxygen, BOD_5, carbonates, bicarbonates, alkalinity, chlorides, hardness, Ca^{++}, Mg^{++}, total Kjeldahl nitrogen, NO_3-N, NO_2-N, NH_4-N, o-PO_4, SO_4, turbidity and TDS) and analysed following the methods by APHA (1998). Temperature, pH and salinity were recorded daily. Net and gross primary productivity (NPP and GPP) were determined using light and dark bottle technique (APHA 1998).

Determination of chlorophyll a, pheophytin a and plankton biomass from pond water

Four replicate water samples (10 L for each sample) from each experimental pond were collected at an interval of 45 days for determining the chlorophyll *a* and phaeophytin *a* contents. The water samples were filtered through Whatman Filter Paper No. 40 and extracted using cold acetone before their chlorophyll a and phaeophytin a contents were determined spectrophotometrically (APHA 1998).

Plankton samples were also collected by passing 20 L of water taken from five different locations (4 L from each location) of each pond through plankton net (mesh size 125 µm) at an interval of 45 days. Each of the plankton samples were carefully transferred to a measuring cylinder diluted with 50 ml of distilled water and preserved with 5 per cent buffered formalin in a small plastic bottle (concentrated sample). Plankton numbers were estimated using the Sedgwick Rafter cell. One ml of the concentrated sample was placed on to the counting chamber and left to stand for 5 minutes to allow plankton to settle. Ten randomly selected fields of the chamber were counted under a binocular microscope and plankton density was calculated using the following formula:

$$\text{Plankton (numbers} \bullet L^{-1}) = 100[(\text{No. counted in 10 fields})(\text{Conc. vol. of sample in ml})]/\text{Volume of filtered pond water in L}$$

Identification of plankton to genus level was carried out using the keys of Ward and Whipple (1959), Prescott (1962) and Bellinger (1992).

Sediment quality

Sediment samples in replicate of four from each treatment were collected only once at the end of experimental schedule using a cone sam-

pler (area 858.3 cm^2) and analysed for physico-chemical (moisture, organic matter, pH, salinity, EC, chlorides, alkalinity, o-PO4 and NO_3-N) and biological characteristics (benthic population) following Piper (1966).

Fish harvesting

Fish in each experimental pond were harvested after 90 days of growing on June 20, 2004. The fish were collected after water in the pond was completely drained. The total length (cm) and body weight (g) of the harvested fish were taken as well as their specific growth rate (SGR), condition factor (k) and length-weight relationship (LWR) were calculated as follows.

Condition factor (k) was calculated using following formula:

$$K= [(Wt.) (10^5)]/ L^3$$

Where, Wt. = weight in grams and L = total length in millimeters.

Specific growth rate (% BW•day^{-1}) was calculated using the following formula:

$$SGR (\% BW \bullet day^{-1}) = (In\ Wt.\ f - In\ Wt.\ i) \times 100/t$$

Where, Wt.i and Wt.f denote initial and final weight (g) of fish, respectively and 't' represents time (days).

Length-weight relationship (LWR) of fish was calculated according to the following equation:

$W = c\ L^n$ (Logarithmic form of equation is : $\log W = \log c + n \log L$)

Where, W = weight in kg, c = constant, n = exponential value of length and L = length of fish in cm.

Plankton species diversity $\overline{(d)}$ was determined using the diversity index formula of Shannon and Weaver (Washington 1984).

$$\overline{d} = -\Sigma\ ni/N)\ \log_2\ (ni/N)$$

Where, $\overline{d}$ = species diversity, ni = no. of individuals of i^{th} species, and N = total no. of individuals.

Determination of VSI, HSI and other biochemical estimations

From each treatment, eight fish were obtained and kept on ice tray, viscera and liver of the fish were extirpated for the determination of viscero-somatic index (VSI) and hepato-somatic index (HSI) respectively. Liver and muscle were processed for the estimation of glycogen (Thim-

maiah 1999). Muscle protein was estimated following Lowry et al. (1951). Intestine was extirpated and processed for the determination of protease (Walter 1984), amylase (Sawhney and Singh 2000), cellulolytic (Sadasivam and Manickam 1996) and lipase activity (Thimmaiah 1999).

Proximate composition of fish and feed

Fish carcass (initial and final) and supplementary feed were analysed following AOAC (1995). Dry matter after desiccation in an oven (at 105°C for 24 hours), ash (incineration at 550°C for 4 hours in a muffle furnace), nitrogen using micro-Kjeldahl method were determined and the crude protein content was estimated by multiplying nitrogen by a factor of 6.25. Crude fat was determined by petroleum ether extraction (Soxhlet's apparatus). Carcass phosphorus was determined spectrophotometrically after acid digestion (nitric acid:perchloric acid 10:1). Per cent nitrogen free extract (NFE) was calculated by substracting the sum of per cent crude protein, crude fat, ash, moisture (% wet weight) and crude fibre from 100. Energy content of fish, fish feed and periphyton were calculated using the average caloric conversion factors of 0.3954, 0.1715 and 0.2364 $kJ \bullet g^{-1}$ for lipid, carbohydrate and protein, respectively (Henken et al. 1986).

Statistical analysis

The data were subjected to ANOVA to test the effect of treatment using the following model:

$Y_{ij} = \square + T_i + e_{ij}$

$Y_{ij} = j^{th}$ observation of i^{th} treatment

$\square$ = overall mean

T_i = effect due to i^{th} treatment

e_{ij} = random error NID (o, $\square^2$)

Arcsine transformation of the data presented in percentage was done before analysis of variance. Means were compared using Tukey's test as described by Snedecor and Cochran (1982). Coefficient of correlation between different parameters and multiple regression between independent (hydrochemical parameters) and dependent (biological and productivity parameters) was determined.

Results

Fish growth

Fish survival varied between 90 and 95%. ANOVA showed a significant ($P<0.05$) increase in mean fish weight, length, biomass and growth rates (growth per day and SGR) including condition factor in ponds provided with substrate (substrate ponds). Mean fish weight increased from 1.47 ±0.09 to 196.8 ±5.57 g in substrate ponds ($F = 30.61$, d.f. 2,147, $P<0.0001$) in comparison with feed ponds (from 1.59 ±0.08 to 150.6 ±5.14 g) and control ponds (from 1.41 ±0.08 to 126.0 ±5.29) (Table 1) indicating an increase of about 31% growth rate due to the provision of substrate over feed ponds and about 56% over control ponds. SGR was 5.4 in substrate ponds compared to 5.1 and 5.0 in feed ponds as well as in controls, respectively. The mean length of Nile tilapia in ponds with substrate was 21.2 cm compared to 19.2 cm in feed ponds and 18.9 cm in controls. LWR indicates that the value of 'n' was also higher in ponds provided with substrate (n=3.1), followed by feed ponds (n=3.0) and controls (n=2.9). No significant variations in the values of 'k' (condition factor) were observed among different treatments.

Fish carcass

Carcass composition had revealed significantly ($P<0.05$) higher accumulation of protein, fat, phosphorus and energy in fish grown in ponds provided with substrate in comparison with other treatments. Significantly ($P<0.05$) lower values in these parameters were observed in fish carcass grown in control ponds. No differences in ash contents among the different treatments were observed (Table 1).

Physico-chemical characteristics of water

Water salinity during the experimental period fluctuated between 12.8-13.5 ppt., and pH values at 7.75-8.71 indicating that the pond waters were well buffered, with EC values ranging 17.4-19.0 dSm^{-1}. No significant ($P<0.05$) variation in dissolved oxygen (DO), carbonates, hardness, chlorides and total kjeldahl nitrogen were observed among different treatments. Free carbon dioxide was absent during the entire period of investigations. Turbidity, NH_4-N, NO_2-N, SO_4 and BOD_5 remained significantly ($P<0.05$) lower, whereas NO_3-N and magnesium remained significantly ($P<0.05$) higher in substrate ponds in comparison with other treatments. A review of the data further indicates that productivity indicating parameters

Table 1. Effect of substrate and supplementary feeding on growth performance and carcass composition of *Oreochromis niloticus* (duration of experiment –90 days)

Treat-ments	INITIAL FISH STOCK			FINAL FISH STOCK (after 90 days)			Increase in mean fish wt. (g) (Mean length, cm)	Cf (k)	SGR (% BW• day^{-1})	LWR 'n'
	Stocking density 375•m^{-2}	Total biomass (kg)	Mean fish weight (g) (Length, cm)	Survival (%)	Mean total biomass (kg)	Mean fish wt. (g) (Length, cm)				
Substrate ponds	375	0.55±0.04	1.47±0.09 (4.45±0.08)	95.0^{A}	70.1±1.93^{A}	196.8±5.57^{A} (21.2±0.20)	195.4^{A} (16.7)	2.1±0.06^{A}	5.4±0.06^{A}	3.1
Feed ponds	375	0.59±0.03	1.59±0.08 (4.22±0.09)	91.0^{B}	51.4±1.75^{B}	150.6±5.14^{B} (19.2±0.20)	149.1^{B} (15.0)	2.1±0.05^{A}	5.1±0.06^{B}	3.0
Controls	375	0.53±0.03	1.41±0.08 (4.32±0.09)	90.0^{B}	42.6±1.79^{C}	126.0±5.29^{C} (18.9±0.30)	124.6^{C} (14.6)	2.0±0.06^{A}	5.0±0.06^{B}	2.9

Carcass composition of fish (% wet weight)

Treatment	n	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Phosphorus(%)	Energy (kJ g^{-1})
Initial	4	70.9±0.20^{A}	15.8±0.22^{D}	3.1±0.17^{C}	3.0±0.16^{B}	0.5±0.02^{D}	6.1±0.03^{C}
Substrate ponds	4	67.5±3.08^{B}	18.5±0.22^{A}	3.4±0.17^{A}	3.2±0.04^{AB}	1.1±0.01^{A}	7.0±0.07^{A}
Feed ponds	4	67.6±0.55^{B}	17.6±0.18^{B}	3.3±0.04^{AB}	3.0±0.07^{B}	0.9±0.01^{B}	6.9±0.07^{A}
Controls	4	67.6±0.20	17.1±0.16^{C}	3.1±0.26^{BC}	3.3±0.03^{A}	0.7±0.01^{C}	6.8±0.08^{B}

All values are mean±SE of mean, n=number of observations

Means bearing different superscripts in the same column differ significantly ($P<0.05$)

(turbidity and total alkalinity), nutrients (NO_2-N, o-PO_4 and sulphate), bicarbonate, calcium, TDS and BOD_5 were significantly ($P<0.05$) higher in feed ponds in comparison with controls and substrate ponds (Table 2).

Table 2. Mean values of physico-chemical characteristics of water recorded from different treatment ponds stocked with *Oreochromis niloticus* (duration of experiment-90 days)

Parameters	n	Treatments			LSD
		Substrate ponds	Feed ponds	Control	
EC ($dS•m^{-1}$)	16	$17.9±0.09^{B}$	$17.4±0.30^{B}$	$19.0±0.26^{A}$	0.67
pH	-	7.81–8.30	7.75–8.28	7.97–8.71	-
Dissolved oxygen ($mg•L^{-1}$)	16	$5.7±0.21^{A}$	6.7±0.46	$6.7±0.30^{A}$	0.96
BOD_5 ($mg•L^{-1}$)	16	$3.7±0.12^{B}$	$4.6±0.11^{A}$	$4.0±0.12^{B}$	0.34
Carbonates ($mg•L^{-1}$)	16	$5.5±0.50^{A}$	$4.8±0.51^{A}$	$4.3±0.31^{A}$	1.28
Bicarbonates ($mg•L^{-1}$)	16	$199.5±4.82^{B}$	$229.1±1.17^{A}$	$207.0±1.80^{B}$	8.73
Total alkalinity ($mg•L^{-1}$)	16	$205.0±4.44^{B}$	$233.9±1.57^{A}$	$210.6±1.78^{B}$	8.28
Chlorides ($mg•L^{-1}$)	16	$5713.1±9.07^{A}$	$5777.6±10.64^{A}$	$5901.9±10.64^{A}$	28.90
Total hardness ($mg•L^{-1}$)	16	$3350.0±56.27^{A}$	$3350.0±42.81^{A}$	$3300.0±25.82^{A}$	123.79
Calcium ($mg•L^{-1}$)	16	$470.5±6.88^{C}$	$559.8±8.33^{A}$	$517.8±6.33^{B}$	20.61
Magnesium ($mg•L^{-1}$)	16	$530.8±12.09^{A}$	$476.3±13.77^{B}$	$488.2±6.71^{B}$	32.10
Total Kjeldahl nitrogen ($mg•L^{-1}$)	16	$7.2±0.60^{A}$	$8.8±0.73^{A}$	$8.4±0.67^{A}$	1.91
NO_3-N ($mg•L^{-1}$)	16	$1.1±0.01^{A}$	$0.8±0.01^{B}$	$0.7±0.01^{B}$	0.03
NO_2-N ($mg•L^{-1}$)	16	$1.0±0.04^{C}$	$1.3±0.04^{A}$	$1.0±0.01^{B}$	0.03
NH_4-N ($mg•L^{-1}$)	16	$0.7±0.03^{B}$	$1.1±0.06^{A}$	$1.0±0.01^{A}$	0.04
o-PO_4 ($mg•L^{-1}$)	16	$0.2±0.01^{B}$	$0.3±0.01^{A}$	$0.2±0.01^{B}$	0.02
SO_4 ($mg•L^{-1}$)	16	$119.6±5.1^{B}$	$142.8±10.41^{A}$	$126.6±1.91^{AB}$	4.18
Turbidity (NTU)	16	$19.3±0.67^{C}$	$28.8±1.07^{A}$	$25.4±0.64^{B}$	2.33
TDS ($mg•L^{-1}$)	16	$7647.5±80.88^{B}$	$8926.3±146.22^{A}$	$8159.4±138.06^{B}$	356.46

All values are mean±SE of mean of eight replicates and two sampling dates (n=16). Means bearing different superscripts in the same row differ significantly ($P<0.05$). Water temperature during the experimental period ranged between 24.8-31.5°C and water salinity fluctuated between 12.8–13.5 ppt. LSD=Least significant difference

Biological characteristics of pond water

Net primary productivity (NPP) and chlorophyll *a* were significantly ($P<0.05$) higher in feed and control ponds in comparison with substrate ponds. No significant ($P<0.05$) variations in pheophytin *a* values among different treatments were observed (Table 3). Epilithic periphyton density, chlorophyll *a*, and pheophytin *a* and gross primary productivity

(GPP) were also significantly ($P<0.05$) higher in feed ponds in comparison with controls and substrate ponds.

Table 3. Mean values of hydrobiological characteristics and pond sediment recorded from different treatment ponds stocked with *Oreochromis niloticus* (duration of experiments–90 days)

Parameters	n	Treatments			LSD
		Substrate ponds	Feed ponds	Control	
Hydrobiological characteristics:					
Net primary productivity (NPP) (mg C l^{-1} d^{-1})	16	0.97 ± 0.05^{B}	1.0 ± 0.04^{A}	0.9 ± 0.03^{A}	0.13
Gross primary productivity (GPP) (mg C l^{-1} d^{-1})	16	2.3 ± 0.08^{B}	2.5 ± 0.09^{A}	2.3 ± 0.03^{B}	0.31
Chlorophyll *a* (µg l^{-1})	16	2.2 ± 0.13^{B}	3.3 ± 0.26^{A}	2.8 ± 0.22^{A}	0.59
Pheophytin *a* (µg l^{-1})	16	1.3 ± 0.14^{A}	1.3 ± 0.15^{A}	1.0 ± 0.08^{A}	0.36
Phytoplankton (numbers l^{-1})	16	8.0×10^{3} $\pm842.0^{C}$	18.0×10^{3} $\pm406.0^{A}$	16.0×10^{3} $\pm1180.0^{B}$	3700.10
Zooplankton (numbers l^{-1})	16	9.0×10^{3} $\pm1001.0^{B}$	14.0×10^{3} $\pm629.0^{A}$	9.0×10^{3} $\pm798.0^{B}$	2346.50
Phytoplankton d	16	1.1 ± 0.15^{C}	1.7 ± 0.15^{B}	2.2 ± 0.04^{A}	0.35
Zooplankton d	16	1.8 ± 0.04^{A}	1.8 ± 0.12^{A}	1.5 ± 0.14^{A}	0.31
Epilithic periphytondensity (numbers cm^{-2})	16	14.3×10^{3} $\pm3248^{B}$	19.4×10^{3} $\pm3072^{A}$	16.0×10^{3} $\pm2155^{B}$	2900.20
Epilithic chlorophyll *a* (µg cm^{-2})	16	9.5 ± 0.97^{B}	12.7 ± 1.78^{A}	10.5 ± 0.66^{B}	1.39
Epilithic pheophytin *a* (µg cm^{-2})	16	3.5 ± 0.41^{B}	5.0 ± 0.24^{A}	3.1 ± 0.56^{B}	1.01
Pond sediment characteristics:					
Moisture (%)	6	42.0 ± 0.63^{A}	41.5 ± 0.26^{A}	41.2 ± 0.19^{A}	1.29
Organic matter (%)	6	1.4 ± 0.02^{C}	1.6 ± 0.01^{A}	1.5 ± 0.01^{B}	0.06
pH	6	8.6 ± 0.01^{A}	8.6 ± 0.00^{A}	8.6 ± 0.00^{A}	0.03
Salinity (ppt)	6	0.4 ± 0.02^{A}	0.4 ± 0.04^{A}	0.4 ± 0.02^{A}	0.10
EC (dSm^{-1})	6	1.7 ± 0.03^{A}	1.9 ± 0.01^{A}	1.8 ± 0.02^{A}	0.45
Chlorides (mg g^{-1})	6	10.1 ± 0.35^{A}	10.2 ± 0.30^{A}	9.5 ± 0.45^{A}	1.37
Alkalinity (mg g^{-1})	6	1.7 ± 0.03^{B}	1.9 ± 0.01^{A}	1.8 ± 0.03^{A}	0.09
o-PO_4 (mg g^{-1})	6	0.003 ± 0.003^{A}	0.004 ± 0.002^{A}	0.004 ± 0.0004^{A}	0.001
NO_3-N (mg g^{-1})	6	0.33 ± 0.001^{C}	0.04 ± 0.001^{A}	0.04 ± 0.001^{B}	0.002
Benthos (numbers m^{-2})	6	2600.0 ± 81.65^{B}	3000.0 ± 115.47^{A}	2600.0 ± 115.47^{B}	337.22

All values are mean±SE of mean of eight replicates and two sampling dates (n=16), Sediment values are mean±SE of mean of six observations (n=6). Means bearing different superscripts in the same row differ significantly ($P<0.05$). Water temperature during the experimental period ranged between 24.8-31.5°C. LSD=Least significant difference.

Phytoplankton density was higher in feed ponds and controls in comparison with substrate ponds. With regard to zooplankton population, significantly ($P<0.05$) higher values were observed in feed ponds in comparison with other treatments. On the other hand, phytoplankton species diversity was lower in substrate ponds, while not much variation in zooplankton species diversity were observed among different treatments (Table 3).

Sediment characteristics

No significant variations in moisture, pH, electrical conductivity (EC), salinity, chlorides and orthophosphate were observed among different treatments. However, alkalinity, NO_3-N, organic matter and benthos were significantly ($P<0.05$) higher in feed ponds compared to other treatment ponds (Table 3).

Periphyton and pigment concentrations

Mean periphyton density increased with increase in depth up to 50 cm, which declined thereafter. Peak values in periphyton density at most of the depths were observed during weeks 8-12 (Figure 1). Significantly ($P<0.05$) higher values for mean dry matter (DM), ash free dry matter (AFDM), ash, ash percentage of dry matter, pigment concentrations (chlorophyll *a* and pheophytin *a*), mean peripyton productivity and algal constitutes of periphyton were also observed at 50 cm substrate depth. Variations in chlorophyll *a* and pheophytin *a* concentrations had revealed peak values at 50 cm depth during weeks 2-6 (Figure 2) and weeks 2, 8 and 12 (Figure 3), respectively. Autotrophic index (AI) decreased with increase in substrate depth from 0-50 cm and thereafter at 75 cm depth an increase was observed (Table 4).

Irrespective of the treatments, the plankton communities principally consisted of two groups of phytoplankton (chlorophyceae and bacillariophyceae) and two groups of zooplankton (rotifera and copepoda). Phytoplankton were represented by 8 taxa, 4 belonging to bacillariophyceae and 4 to chlorophyceae. Succession studies showed that *Closterium* (chlorophyceae), *Synedra* and *Navicula* (bacillariophyceae) formed the stable community. Zooplankton were represented by 5 taxa, 2 belonging to copepoda and 3 to rotifera. Succession studies showed that *Cyclops* and *Nauplius* (copepoda), *Polyarthra* and *Brachionus* (rotifera) formed the stable community.

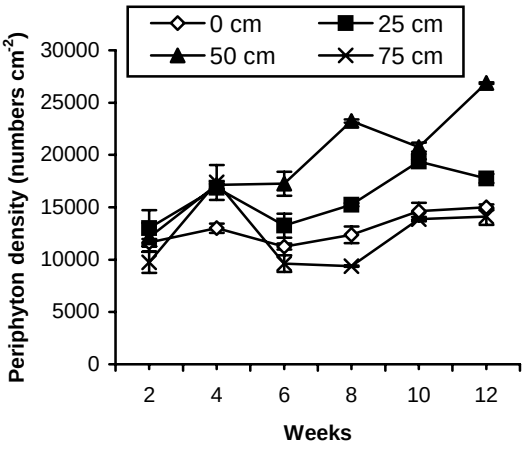

Figure 1. Fortnightly variations in mean values (±SE of mean, n=48) of periphyton density (numbers•cm^{-2}) at different depths (0, 25, 50 and 75 cm) from ponds provided with additional substrate and stocked with *O. niloticus* under monoculture

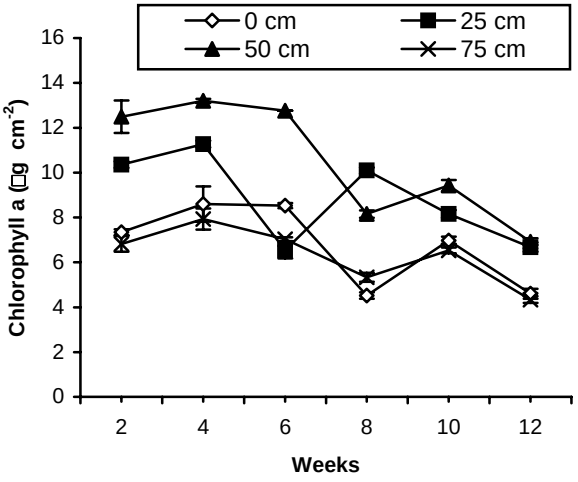

Figure 2. Fortnightly variations in mean values (±SE of mean, n=48) of chlorophyll *a* (periphyton) concentrations (□g•cm^{-2}) at different depths (0, 25, 50 and 75 cm) from ponds provided with additional substrate and stocked with *O. niloticus* under monoculture

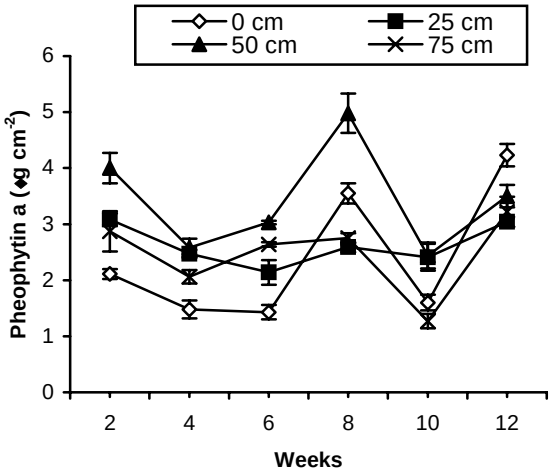

Figure 3. Fortnightly variations in mean values (±SE of mean, n=48) of pheophytin *a* (periphyton) concentrations (□g•cm^{-2}) at different depths (0, 25, 50 and 75 cm) from ponds provided with additional substrate and stocked with *O. niloticus* under monoculture

Digestive enzyme activity, muscle protein, muscle glycogen, liver glycogen, viscero-somatic index (VSI) and hepato-somatic index (HSI)

The ability of the fish to use nutrients depends upon several factors which include availability of digestive enzymes in appropriate amounts. The results of the present studies further depict that total (protease, amylase and cellulolytic) and specific (amylase, cellulolytic and lipase) enzyme activities remained significantly ($P<0.05$) higher, while specific protease activity was lower in fish grown in ponds provided with substrate, followed by feed and control ponds. No significant ($P<0.05$) variations in lipase activity among different treatments were observed (Table 5). Similarly, muscle protein, muscle glycogen, VSI (indicating better fat accumulation) and HSI (hepato-somatic index) values were also significantly ($P<0.05$) enhanced in fish grown in ponds provided with substrate, followed by feed ponds and controls (Table 5), while no variations in liver glocygen levels among different treatments were observed.

Table 4. Periphyton dry matter (DM), ash free dry matter (AFDM), ash contents, periphyton number, total pigment concentration, chlorophyll *a*, pheophytin *a* and autotrophic index (AI) at different depths from substrate ponds stocked with *Oreochromis niloticus* (duration of experiment–90 days)

Parameters	n	Depths (cm)			
		0	25	50	75
Dry matter (DM) (mg•cm^{-2})	2	1.4±0.01^{B}	1.6±0.01^{B}	1.7±0.03^{A}	1.1±0.05^{C}
AFDM (mg•cm^{-2})	2	0.9±0.02^{AB}	0.9±0.03^{AB}	0.9±0.06^{A}	0.7±0.01^{B}
Ash (mg•cm^{-2})	2	0.5±0.01^{BC}	0.6±0.02^{B}	0.8±0.03^{A}	0.4±0.04^{C}
Ash (% of DM)	2	39.0±0.90^{AB}	42.5±1.77^{AB}	47.5±1.77^{A}	37.5±1.77^{B}
Periphyton number (units•cm^{-2})	48	12979±577^{C}	15917±947^{B}	19562±1937^{A}	12354±1222^{C}
Total pigment concentration (□g•cm^{-2})	48	9.0±0.40^{C}	11.6±0.80^{B}	13.9±0.91^{A}	8.9±3.60^{C}
Chlorophyll *a* (□g•cm^{-2})	48	6.8±0.68^{BC}	8.9±0.75^{B}	10.5±0.10^{A}	6.3±0.48^{C}
Pheophytin *a* (□g•cm^{-2})	48	2.4±0.45^{C}	2.6±0.13^{B}	3.4±0.36^{A}	2.5±0.26^{BC}
Autotrophic index (AI)	-	127.2	97.3	85.8	107.4
Algal constitute of periphyton biomass (%)	-	32-41	39-52	41-53	37-48
Periphyton productivity (mg ash free weight•m^{-2}•day^{-1})	-	0.6	0.7	0.9	0.5

All values are mean± S.E. of mean. Means bearing different superscripts in the same row differ significantly (P<0.05).

Table 5. Effect of additional substrate and supplementasry feeding on activity of digestive enzymes, muscle protein, muscle glycogen, liver glycogen, viscero-somatic index and hepato-somatic index of *Oreochromis niloticus* grown in inland saline groundwater ponds

Parameters	n	*Oreochromis niloticus*			LSD
		Substrate ponds	Feed ponds	Control	
Total protease enzyme activity ($\mu g \bullet g^{-1} \bullet h^{-1}$)	8	5.3 ± 0.09^{A}	4.1 ± 0.24^{B}	2.8 ± 0.02^{C}	0.48
Specific protease enzyme activity[a]	8	1.8 ± 0.02^{B}	2.0 ± 0.09^{AB}	2.0 ± 0.01^{A}	0.19
Total amylase activity ($mg \bullet g^{-1} \bullet h^{-1}$)	8	2.2 ± 0.08^{A}	1.9 ± 0.03^{B}	1.7 ± 0.01^{C}	0.19
Specific amylase activity[b]	8	1.0 ± 0.02^{A}	1.0 ± 0.01^{A}	0.8 ± 0.02^{B}	0.05
Total cellulolytic activity ($mg \bullet g^{-1} \bullet h^{-1}$)	8	4.1 ± 0.14^{A}	2.7 ± 0.02^{B}	2.2 ± 0.08^{C}	0.32
Specific cellulolytic activity[c]	8	1.9 ± 0.02^{A}	1.4 ± 0.02^{B}	1.0 ± 0.03^{C}	0.08
Total lipase activity ($mg \bullet g^{-1} \bullet h^{-1}$)	8	2.7 ± 0.58^{A}	3.3 ± 0.45^{A}	1.9 ± 0.12^{A}	1.47
Specific lipase activity[d]	8	1.6 ± 0.09^{A}	1.6 ± 0.19^{A}	1.0 ± 0.04^{B}	0.40
Muscle protein ($mg \bullet g^{-1}$)	8	164.0 ± 2.61^{A}	151.3 ± 1.18^{B}	151.3 ± 0.74^{B}	5.53
Muscle glycogen ($mg \bullet g^{-1}$)	8	2.3 ± 0.07^{A}	2.1 ± 0.03^{B}	1.7 ± 0.03^{C}	0.17
Liver glycogen ($mg \bullet g^{-1}$)	8	2.0 ± 0.03^{A}	2.1 ± 0.01^{A}	2.1 ± 0.03^{A}	0.10
Viscero-somatic index	8	12.2 ± 0.61^{A}	10.4 ± 0.33^{B}	9.1 ± 0.26^{C}	1.41
Hepato-somatic index	8	1.5 ± 0.06^{A}	1.3 ± 0.07^{AB}	1.1 ± 0.11^{B}	0.27

All values are mean±SE of mean, n= number of observations. Means bearing different superscripts in the same row differ significantly ($P<0.05$); a = □g of tyrosine liberated/mg of protein/min, b = □g of maltose liberated/mg of protein/min, c = □g of glucose liberated/mg of protein/min, d = micromole fatty acid liberated/mg of protein h^{-1}. LSD = Least significant difference

Discussion

In the present study, fish growth in terms of weight gain, specific growth rate (SGR) was significantly higher in substrate ponds, which resulted in 31% higher in weight-gain compared to feed ponds and 56% compared to controls. High exponential value of 'n' of LWR (~3) indicated that *O. niloticus* grown in inland saline groundwater ponds (12.8-13.5 ppt salinity) followed the cube law and thus inland saline groundwater of high

hardness appeared to be conducive for the growth of *O. niloticus*. Jana et al. (2004; 2006) have reported 35.4% higher growth in *Mugil cephalus* and 72.5% higher growth in *Chanos chanos* when grown/cultured in inland saline groundwater ponds provided with substrate for the development of periphyton as compared to controls. Similar effects of periphyton on growth performance of other freshwater fish species (e.g., *Labeo rohita, Labeo gonius*) low in the food chain were also reported by Azim et al. (2001a; 2001b; 2002).

Although provision of substrates did not significantly affect the survival rates of the fish, the substrates appeared to have the effect of reducing stress by acting as a shelter or hiding place for the fish. Without shelter or hiding place, the fish will show elevated swimming activity which may lead to an increase in metabolic expenditure, wasting the energy that can be used for growth. Kuhlmann and Koops (1980) reported that eels kept in silo (with housing) grew much faster than when the eels were kept in boxes with no housing. Wahab et al. (1999a; 1999b) and Keshavanath et al. (2002) have also reported high survival of fish in ponds provided with additional substrate in ponds.

Nile tilapia (*Oreochromis niloticus*) was known to a herbivore (Bowen 1982; Trewavas 1983) grazing on the algal biomass (periphyton) grown on substrates (Getachew 1987). The mixture of algae in the periphyton are likely to be nutritive that could enhance growth of the fish grazing on them as reported by Horn (1989) and Huchette et al. (2000). Kumar et al. (2005) further reported that species of algae from the stomach contents of tilapia (*Oreochromis* spp.) were similar to those in the periphyton indicating that the fish did graze on the periphyton to sustain its growth.

Carcass protein, fat and phosphorus increased significantly ($P<0.05$), while those of moisture and ash decreased in fish grown in substrate ponds as compared with other treatments. These changes correlated well with the growth patterns of the fish kept in different treatments, thus also confirming the suitability of periphyton as feed and its nutritional value. Fish in substrate ponds had high VSI and carcass fat, which showed an inverse relationship with moisture contents.

Only minor differences in water quality parameters were observed among different treatments. pH remained alkaline in all the three treatments. Alkalinity, turbidity, NH_4-N, NO_2-N, o-PO_4, SO_4, BOD_5, epilithic chlorophyll *a* and pheophytin *a* values were significantly ($P<0.05$) higher in treatment 2. The values of these parameters in general were low in ponds provided with additional substrate indicating the utilization of nutri-

ents in periphyton growth. Low ammonia (NH_4-N) levels in ponds provided with additional substrate is also supported by Ramesh et al. (1999) and Jana et al. (2004; 2006) concluded that enhanced bacterial biofilms on substrate might have reduced ammonia levels through the promotion of nitrification. Reduction in NH_4-N and turbidity levels and absorption of particulate organic matter by the periphyton may also be attributed to the biofilter properties of the periphyton.

Plankton density was low in ponds provided with substrate. High chlorophyll *a* concentration also coincided with the phytoplankton population. Azim et al. (2001a), Keshavanath et al. (2001a) and Jana et al. (2004; 2006) reported low values of chlorophyll *a* in ponds provided with substrate, which may be attributed to the incorporation of planktonic flora and fauna in periphyton production. Multiplevariate analysis of data showed a significant ($P<0.05$) positive correlation of NPP with turbidity ($r=0.37$), alkalinity ($r=0.30$), total Kjeldahl nitrogen ($r=0.30$), o-PO_4 ($r=0.34$) and chlorophyll *a* ($r=0.40$), clearly revealing that ponds were in high trophic status.

A review of sediment chemistry indicate high values of NO_3-N, alkalinity, organic matter and benthic population in treatment 2, where the fish were fed on a supplementary diet. Low values of organic matter in the pond sediment with substrate ponds may be attributed to its incorporation in periphyton. High benthic population in feed ponds (treatment 2) may be attributed to the availability of sufficient organic matter (as result of feed) which remained unconsumed.

Periphyton biomass measured in terms of periphyton density, DM, AFDM and pigment concentrations (chlorophyll *a* and pheophytin *a*) increased significantly ($P<0.05$) with depth up to 50 cm, a decline thereafter in their values indicates that the euphotic zone was only up to 50 cm. These findings are in accordance with those of Konan-Brou and Guiral (1994), Azim et al. (2001b) and Keshavanath et al. (2001b) and also with the recent studies of Jana et al. (2004) on *Mugil cephalus* and *Chanos chanos*.

High ash content (39.0-47.5%) of periphyton recorded in the present studies may be attributed to the suspended particles entrapped by the periphyton community. Low turbidity in ponds provided with substrate in comparison to other treatments further supports this view. The algal content of the periphyton can be deduced from the relationship between ash free dry matter (AFDM) and chlorophyll *a* concentrations. The periphyton autotrophic index (AI) values showed that 1.0 mg of chlorophyll *a* is

equivalent to about 127.2, 97.3, 85.8 and 107.4 mg of AFDM at 0, 25, 50, and 75 cm depths, respectively indicating high autotrophic association in periphyton at the surface. Huchette et al. (2000) and Azim et al. (2001a) reported that AI values fluctuated between 150 to 300 and 190 to 350, respectively under ungrazed conditions. In the present studies, due to grazing pressure exerted by the stocked fish, autotrophic index values remained low and ranged between 85.8-127.2. AI values decreased with increase in substrate depth from 0-50 cm and thereafter at 75 cm an increase was observed, which may be attributed to low penetration of light (beyond euphotic zone) resulting in low autotrophic association and an increase in AI values. These results are similar to those reported by Azim et al. (2001a). The decrease in AI values may be due to an increase in productivity in terms of chlorophyll *a* at 50 cm depth. The increase in chlorophyll *a* concentration may be attributed to rejuvenation of autotrophs due to constant grazing by the fish at this depth. Hatcher (1983), Hay (1991) and Huchette et al. (2000) also stated that productivity increases when periphyton community are constantly grazed by the fish. If one mg of chlorophyll *a* can be derived from 65-85 mg algal dry matter (Reynolds 1984; Dempster et al. 1993), this may indicate that algae constitutes 32-41, 39-52, 41-53 and 37-48% of periphytic biomass at different depths (0, 25, 50, 75 cm, respectively). The bulk of the periphyton (47-68%) in the present study is thus not of an algal nature, confirming the importance of periphyton for attracting heterotrophs and trapping organic matter.

Digestive enzyme activities in fish respond to changes in the quality and quantity of nutrients intake (Coway et al. 1981). High intestinal enzyme activity coupled with high growth has been observed in fish grown in ponds provided with additional substrate, followed by treatment where the fish were fed on supplementary feed and controls where, neither any additional substrate was provided nor the fish were fed on any supplementary feed. Studies of Verdegem et al. (2001) on proximate composition of periphyton had revealed significantly ($P<0.05$) high protein (41.4%), fat (7.9%) and energy (21.2 $kJ \bullet g^{-1}$) content in samples collected from ponds without fish (ungrazed conditions) as compared to the periphyton samples obtained from ponds stocked with fish (grazed conditions), indicating the high nutritive value of periphyton, reflecting the metabolic interaction and adjustments, which were sustained by the nutritional status of periphyton in treated ponds. High HSI values and high accumulation of muscle protein, carcass protein and muscle glycogen also support high growth in fish grown in ponds provided with additional substrate. Fish maintained in substrate ponds also had high accumulations of fat as indicated by high

VSI and carcass fat contents. These studies thus indicate that periphyton possess high nutritive value required for fish growth.

The findings of this study thus indicate that periphyton supported production technology offers considerable potential for enhancing aquaculture production without any deleterious impact on pond ecosystem. Significantly higher weight gain of fish in ponds provided with substrate as compared to feed ponds where the fish were fed on supplementary diet, is a clear indication of economic viability of the technology.

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